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16. ABSTRACT

This report is an assessment of the field sampling methodologies used to measure concentrations of airborne bacteria and viruses in and around waste handling and processing facilities.

The sampling methods are discussed as well as the problems encountered and subsequent changes made to improve the methods. Comparisons are also made to preliminary recommendations from ASTM subcommittee E-38.07 on Health and Safety Aspects of Resource Recovery plants.

The results showed air filter methods such as a Hi-Vol sampler are not generally recommended because of dessication effects of the air stream passing over the filter. AGI-30 impingers and Andersen agar plate impactors are preferred methods. The complete tests are fully reported in the final report, "Assessment of Bacteria and Virus Emissions at a Refuse Derived Fuel Plant and Other Waste Handling Facilities," EPA 600/2-78-152, U.S. Environmental Protection Agency, August 1978.

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COMPARISON OF METHODS FOR SAMPLING BACTERIA AT SOLID WASTE PROCESSING FACILITIES

by

P. G. Gorman
D. E. Fiscus
M. P. Schrag
L. J. Shannon
Field Programs Section
Midwest Research Institute
Kansas City, Missouri 64110

Contract No. 68-02-1871

Project Officer

Carlton C. Wiles
Solid and Hazardous Waste Research Division
Municipal Environmental Research Laboratory
Cincinnati, Ohio 45268

MUNICIPAL ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OHIO 45268

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FOREWORD

The Environmental Protection Agency was created because of increasing public and government concern about the dangers of pollution to the health and welfare of the American people. Noxious air, foul water, and spoiled land are tragic testimony to the deterioration of our natural environment. The complexity of that environment and the interplay between its components require a concentrated and integrated attack on the problem.

Research and development is that necessary first step in problem solution, and it involves defining the problem, measuring its impact, and searching for solutions. The Municipal Environmental Research Laboratory develops new and improved technology and systems for the preservation and treatment of public drinking water supplies and to minimize the adverse economic, social, health, and aesthetic effects of pollution. This publication is one of the products of that research, a most vital communications link between the researcher and the user community.

In St. Louis, the City of St. Louis, Union Electric Company, and the Environmental Protection Agency first demonstrated the use of solid waste as a supplementary fuel in coal-fired power plant boilers for generating electricity. In addition to the demonstration, research tasks were conducted to evaluate the relative levels of airborne bacteria and viruses at the St. Louis Refuse Processing Plant and other waste handling facilities for purposes of comparison.

This report was prepared as an evaluation of the field sampling methodologies used. The total research program is fully discussed in the report, "Assessment of Bacteria and Virus Emissions at a Refuse Derived Fuel Plant and Other Waste Handling Facilities," EPA-600/2-78-152, U.S. Environmental Protection Agency, August 1978.

Francis T. Mayo, Director
Municipal Environmental Research
Laboratory

ABSTRACT

This report presents an assessment of field sampling methodologies used to measure airborne bacteria and viruses at waste handling facilities. Discussed are the sampling methods used, the problems encountered with the methodology, and the subsequent changes made to improve the methods. ASTM subcommittee E-38.07 on Health and Safety Aspects of Resource Recovery has made preliminary recommendations for airborne microbiological sampling in and around waste handling and processing facilities. This report also presents a comparative discussion of these preliminary ASTM methods.

The results showed that air filter methods such as a Hi-Vol sampler are not generally recommended because of dessication effects of the air stream passing over the filter. AGI 30 impinger samplers and Andersen agar plate impactors are preferred methods. A complete discussion of the entire research program is contained in the report entitled "Assessment of Bacteria and Virus Emissions at a Refuse Derived Fuel Plant and Other Waste Handling Facilities," EPA-600/2-78-152, U.S. Environmental Protection Agency, August 1978.

This report was submitted in fulfillment of Contract No. 68-02-1871 by Midwest Research Institute under the sponsorship of the U.S. Environmental Protection Agency.

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This report was prepared by Midwest Research Institute for the Municipal Environmental Research Laboratory, U.S. Environmental Protection Agency, Cincinnati, Ohio, under Environmental Protection Agency Contract No. 68-02-1871. The project officer for the Environmental Protection Agency was Mr. Carlton C. Wiles. Its purpose is to compare the field sampling techniques and results of work performed by Midwest Research Institute to assess airborne bacterial and viral concentrations at solid waste handling and processing facilities. The field sampling techniques used are described in detail along with tentative recommendations made by the ASTM E-38.07 Subcommittee on Health and Safety Aspects of Resource Recovery. This report was written by Messrs. P. G. Gorman, D. E. Fiscus, M. P. Schrag, and Dr. L. J. Shannon.

SECTION 1

INTRODUCTION

The energy shortage of recent years and the increasing unpopularity of solid waste disposal via landfills have stimulated unprecedented interest in material and energy recovery from wastes. This interest is reflected in the large number and variety of resource recovery systems being developed. Along with this development comes the need for evaluating such systems to identify their potential environmental impacts and to insure that they are environmentally acceptable. The required sampling and analysis procedures for evaluation of these novel systems are not well developed, and several important testing procedures are just now evolving. One such procedure is field sampling for airborne bacterial and viral emissions from refuse derived fuel (RDF) processing plants, both in the ambient atmosphere and in the air stream from pollution control systems.

Midwest Research Institute (MRI) conducted a bacterial and viral assessment at St. Louis/Union Electric (St. Louis) (1) in late 1976 and at Houston/Browning Ferris Industries (Houston) in mid-1977 (2).

The purpose of this report is to describe critically the bacterial and viral field sampling methodology used at St. Louis, the problems encountered with the methodology, and subsequent changes made in the methodology for the Houston bacterial and viral tests. Finally, the St. Louis and Houston field sampling results are compared with each other and with data from other studies. Included also is a discussion of the tentative recommendations made by the ASTM E-38.07 Subcommittee on Health and Safety Aspects of Resource Recovery (3).

Other reports concerning microbiological assessments at solid waste facilities are:

- Executive Summary - Assessment of Bacteria and Virus Emissions at a Refuse Derived Fuel Plant and Other Waste Handling Facilities (4).
- Dust and Airborne Bacteria at Solid Waste Processing Plants (5).
- Summary Report of Laboratory Methodology Used in Measurement of Airborne Bacteria and Virus (6).

The following sections of this report present a discussion of the pertinent sampling methods used at St. Louis and a comparison of results for the different methods. Since these considerations led to modifications used in subsequent sampling at Houston, the report presents a comparative analysis of the St. Louis and Houston results along with appropriate comparisons with data from other studies. These other studies are research conducted at the National Center for Resource Recovery (NCRR) pilot plant located in Washington, D.C. (7) and at the Richmond, California, Field Station (8). The interested reader is also referred to work being carried out by the Department of Energy, Ames Laboratory, located at Ames, Iowa. The Ames Laboratory currently has an ongoing study of airborne microorganisms at the City of Ames, Iowa, solid waste recovery facility. Data have not yet been published by Ames Laboratory; therefore, no comparative assessment of their work is included in this report.

SECTION 2

SAMPLING TECHNIQUES FOR AEROSOLIZED MICROORGANISMS AT ST. LOUIS

Tests were carried out in November and early December of 1976 to determine relative bacterial and viral levels at the property lines and at in-plant locations for the St. Louis Refuse Processing Plant* and a number of other related waste handling facilities.

The facilities tested were:

- A municipal incinerator
- The St. Louis Refuse Processing Plant
- A wastewater treatment plant
- A refuse transfer station
- A sanitary landfill

In addition to the above facilities, testing was also carried out in downtown St. Louis. Bacterial levels were also ascertained for a refuse collection packer truck.

Three days of testing were carried out at each of the above facilities at the property lines (one upwind and three downwind) and at three in-plant locations. In addition, supplemental tests were conducted at the RDF plant to evaluatate emissions of particulate trace metals, asbestos, and microorganisms from the air classifier system, and removal efficiency of particulates and microorganisms by a pilot-scale mobile filter unit (baghouse).

The sampling devices used were Hi-Vol ambient air samplers, which provide high sampling rates of approximately 19 liters/sec (40 cfm) for relatively long

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* The St. Louis Refuse Processing Plant was a 272 Mg/day (300 tons/day) test facility that operated from 1972 to 1976. The plant produced RDF which was subsequently co-fired (RDF approximately 10% of total heat input) with coal in a steam generator by the Union Electric Company.

periods of time (6 hr) (1). These were supplemented by Andersen agar plate impactors with backup impingers to obtain information on the size distribution of the bacteria-contained particles and to determine if any viruses penetrated the impactor into the impinger. Greenburg-Smith (GS) impingers were used to sample the inlet and outlet of the pilot-scale mobile filter at St. Louis.

Hi-Vol filters used in St. Louis and at related waste handling facilities were located at the property lines and in-plant. Those located in-plant were equipped with precyclones to remove particles larger than 10 μm, which would be nonrespirable and which might overload the filters. At all of these locations, an Andersen agar plate impactor was used to determine the number of bacteria-containing particles as a function of size.

Since both Hi-Vol and Andersen samplers were used in the St. Louis test program, it is possible to compare results from these two sampling methods. However, the validity of such a comparison is questionable because the sampling rate for the Hi-Vol, 19 liters/sec (40 cfm), is much higher than that for the Andersen, 0.47 liter/sec (1 cfm). More importantly, the Hi-Vol results cover a 6-hr sampling period whereas the Andersen results cover a much shorter period (0.5 to 10 min). Recognizing these differences, however, a comparison of the results does illuminate some aspects of bacterial sampling and analysis, as discussed below.

Representative results for the total bacteria count determined from the Hi-Vol and Andersen agar plate impactor samples taken at in-plant locations are shown in Figure 1. From this figure, it can be seen that the counts per cubic meter were higher for the Hi-Vol samplers than for the Andersen samplers. Since it was expected that substantial die-off might occur on the 6-hr Hi-Vol samples, it was surprising that this phenomenon occurred. However, when one considers what the data from these two types of samples represent, the results are not so surprising.

The Andersen results actually represent viable particles, each of which may contain several bacteria. Hi-Vol samplers also capture viable particles, but in the analysis procedure the Hi-Vol filters are first homogenized with distilled water in a Waring blender. It is possible that this would break up the particles and associated numbers or colonies of bacteria, thereby resulting in higher counts than those obtained by the Andersen sampler even if significant die-off had occurred on the Hi-Vol filters during sampling. Although this explanation has not been confirmed, it is the most probable reason for the higher counts obtained by the Hi-Vols and demonstrates how different sampling and analysis procedures can yield different results.

Another interesting comparison that can be made from the St, Louis test data relates to two types of sampling conducted at the inlet to the pilot-scale mobile fabric filter (baghouse) tested at the RDF plant. Figure 2 is a flow diagram of the mobile fabric filter.

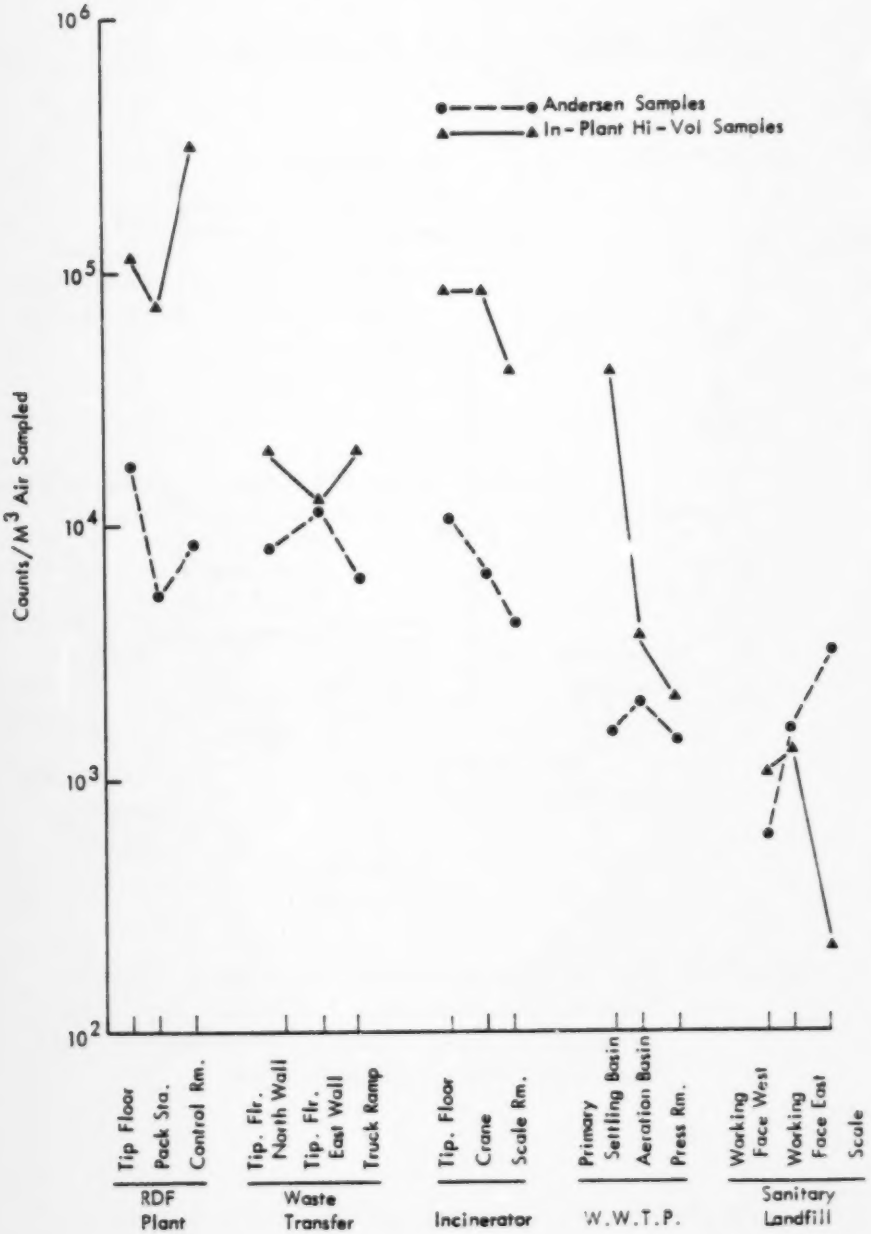
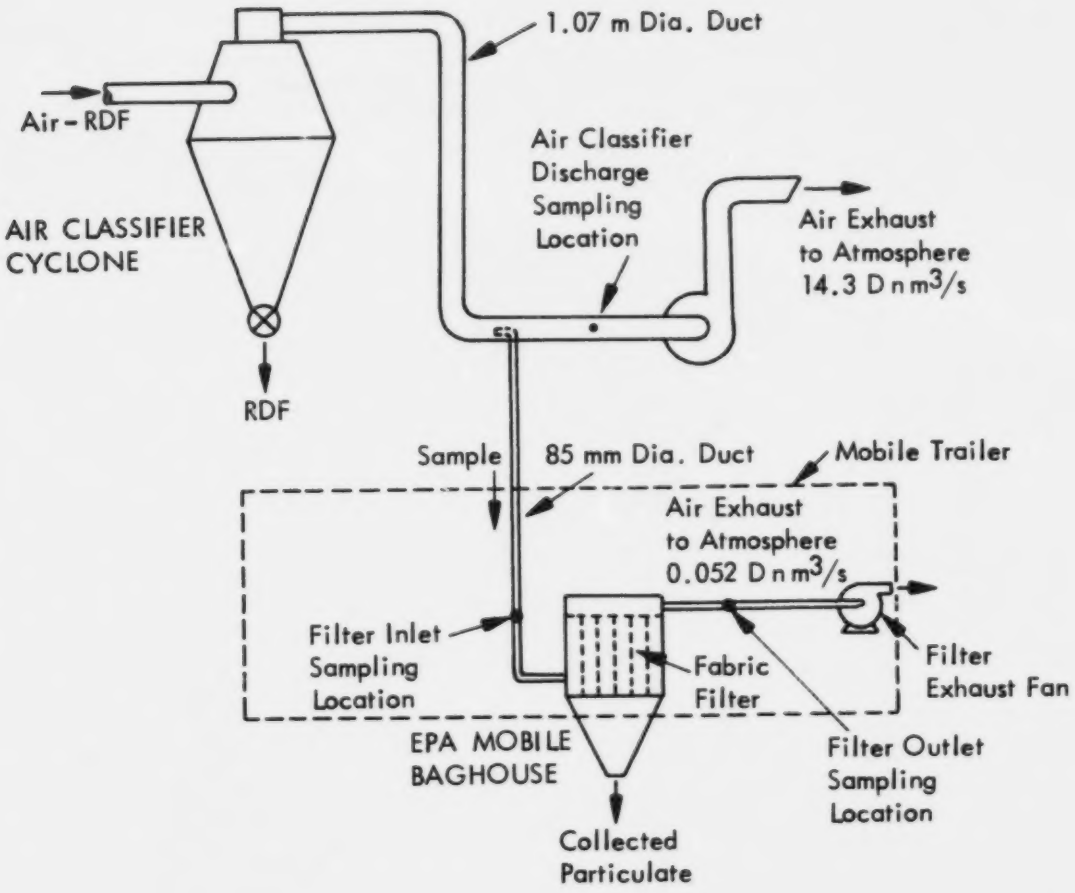


Figure 1. Comparison of Hi-Vol and Andersen bacteria counts.



Operation: EPA bag filter draws a portion of the Air Classifier Cyclone exhaust from the 1.07 m dia. duct. This air sample is passed continuously through the fabric filter to determine filter efficiency.

Figure 2. Flow diagram of EPA mobile baghouse.

Hi-Vol sampling was carried out at the discharge of the air classifier system to determine particulate loading, and samples of the material collected were analyzed for bacteria. This sampling was done at a rate of about 4.7 liters/sec (10 cfm) for approximately 30 min. This sampling was done during the 6-hr period that impinger sampling was also being conducted at the inlet of the mobile baghouse. The latter sampling used a modified GS impinger followed by a standard GS impinger, both of which contained Hanks balanced salt solution (sampling rate, 0.47 liter/sec). Thus, it is possible to compare results from both these sampling techniques as shown in Table 1. Data in this table show that higher counts were obtained from the impinger sampler and may be indicative of increased die-off on the Hi-Vol filters. This die-off rate ranges from 90 to 97%.

In-plant samples taken with the 6-hr Hi-Vols at the St. Louis RDF plant can be compared with results from other studies at the NCRR pilot plant located in Washington, D.C., and the Richmond, California, Field Station. Such a comparison, using representative data, is shown in Table 2. The St. Louis data are based on 6-hr Hi-Vol results whereas the NCRR data (7) are from a Sierra cascade impactor in which paper filter substrates were used. This technique used a low sampling rate of 0.35 liter/sec (0.75 cfm) covering a period of approximately 30 min. Richmond Field Station data (8) were obtained from impinger samplers with nutrient broth (sampling rate and time not specified). Examination of these data, which represent different sampling techniques at different resource recovery plants, shows that the impinger sampling gave higher counts for fecal Streptococcus, total coliforn, and fecal coliform, whereas the 6-hr Hi-Vols gave much lower counts. Again, this may indicate the detrimental effect of long sampling periods (i.e., 6 hr).

It is interesting to note in Table 2 that all three sampling methods showed higher counts for fecal Streptococci than for total coliform or fecal coliform. This supports Peterson's (9) recommendation that alpha hemolytic Streptococcus be used as the indicator of upper respiratory pathogens in air samples from solid waste processing. This organism is usually considered to be human-borne and present in the upper respiratory tract of all normal individuals. Further, it is an easily recognized organism and survives well in air.

At least three studies, including the one at St. Louis, have been carried out at waste processing facilities using the Andersen agar plate impactor. A comparison of the results from these three studies, given in Table 3, shows fairly similar results.

TABLE 1. COMPARISON OF IMPINGER AND HI-VOL SAMPLE RESULTS AT THE AIR CLASSIFIER DISCHARGE

	Concentrations in counts/m^3			
	Total count	Total coliform	Fecal coliform	Fecal Streptococci
Impinger sample	5.25×10^8	3.36×10^6	4.62×10^5	2.25×10^6
Hi-Vol sample	0.12×10^8	$> 0.07 \times 10^6$	0.28×10^5	0.22×10^6

TABLE 2. COMPARISON OF ST. LOUIS RDF PLANT HI-VOL RESULTS WITH TEST DATA FROM OTHER STUDIES

	In-plant bacteria concentrations (counts/m^3)			
Location	Total count	Fecal Streptococci	Total coliform	Fecal coliform
NCRR, Washington, D.C., pilot plant (7)				
Location b[a]	282,000	11,000	1,900	92
Location c[a]	> 1,336,000	> 14,800	1,200	20
St. Louis (1)[b]	4,000-1,630,000	10-478	< 1-> 213	< 1-30
Richmond, California, Field Station (8)	4,700-12,700[c]	14,000-19,000[d]	2,200-3,900[d]	140-2,400[d]

a/ Test data for NCRR are from two test locations in-plant, taken when the plant was operating and samples were not stored overnight. Counts shown are total of all stages of a Sierra impactor, representing particles < 10 μm.

b/ Test data for St. Louis are ranges for in-plant samples, taken with Hi-Vol samplers equipped with a precyclone for removal of particles > 10 μm.

c/ Test data for the Richmond Field Station for total count only are based on sampling with two different impactors (Reynier and Andersen) and three different agars (nutrient agar, plate count agar, and trypticase soy agar).

d/ Test data for the Richmond Field Station for fecal Streptococci, total coliform, and fecal coliform are based on sampling with impingers (AGI) containing nutrient broth. Total count was not determined using impingers.

TABLE 3. COMPARISON OF ANDERSEN AGAR PLATE RESULTS

Test location	In-plant Total count/m^3
Richmond Field Station (8)	11,000-12,700
St. Louis RDF plant (1)	2,075-20,060
Six municipal incinerators (9)	140-27,900

SECTION 3

COMPARISON OF ST. LOUIS AND HOUSTON BAGHOUSE DATA

Testing of baghouses for removal of bacteria has been carried out by MRI at two locations as described in References (1) and (2). Both sampling methods used involved impingers, but with some differences.

Sampling of a pilot-scale baghouse at the St. Louis plant consisted of the following setup. At the inlet, two GS impingers, both containing Hanks' balanced salt solution, were connected in series, but the first was a modified GS impinger intended for capture of large particles that might plug the nozzle in the standard GS impinger. At the outlet, only one standard GS impinger was used. Both the inlet and outlet impingers were operated for 6 hr at a flow rate of 0.47 liter/sec (1.0 cfm).

Later, prior to preparations for the sampling at Houston, a review of the literature indicated that the use of an AGI-30 impinger would be a preferable sampling technique because, unlike the GS impingers, the AGI-30 impingers are designed for microbial sampling. Also, it was determined that gelatin milk would be a more suitable medium for use in the impingers and that sampling time should be reduced to 30 min to decrease the detrimental effect of air on the organisms of interest. Again, however, it was necessary to precede the AGI-30 impinger used on the inlet with a modified GS impinger (containing no solutions) in order to remove any larger particles that would have plugged the small nozzle in the AGI impinger. In sampling airborne microorganisms, May (10) had also used a "pre-impinger" ahead of an impinger to obtain particle size segregation. No analysis was performed on the catch in the GS impinger in the initial tests at Houston, because it was assumed this would not significantly affect the expected high bacterial concentrations. However, the data obtained proved this assumption to be erroneous. The inital results showed lower bacterial concentrations at the inlet of the baghouse than at the outlet. Therefore, in subsequent testing, the GS impinger catch was rinsed out and analyzed; and it showed concentrations of two to three orders of magnitude higher than the catch from the AGI-30 impinger alone. This in itself indicates that most of the bacteria are associated with larger particles.

Based on the second set of tests at Houston, a comparison can be made of the inlet and outlet results in Houston and St. Louis, respectively, as given in Table 4. As can be seen in this table, the inlet particulate concentration

TABLE 4. RESULTS OF IMPINGER TESTS FOR BAGHOUSES AT HOUSTON AND ST. LOUIS

	Average particulate concentration	Bacteria counts/m^3 [a]			
		Total counts	Fecal Streptococci	Total coliform	Fecal coliform
Inlet					
Houston [b]	23.0 g/Nm^3	0.9-75.6 x 10^6	2.5-361 x 10^5	0.4->455 x 10^4	0.4->455 x 10^4
St. Louis [b]	0.3 g/Nm^3	525 x 10^6	22 x 10^5	336 x 10^4	46 x 10^4
Outlet					
Houston [b]	0.003 g/Nm^3	1.9-68.2 x 10^4	4.5-95.5 x 10^3	<91-5,000	<91-3,180
St. Louis [b]	0.00015 g/Nm^3	210 x 10^4	2.1 x 10^3	357	230

[a] Impinger samples from St. Louis and Houston were also analyzed for Staphylococci, Klebsiella sp. and Salmonella, but all were very low or below detectable limits.

[b] Bacterial concentrations shown for Houston are ranges for six 30-min samples taken at a flow rate of 0.08 liter/sec (0.18 cfm), whereas only one sample was obtained from St. Louis, representing one 6-hr test at a sampling rate of 0.47 liter/sec (1.0 cfm).

at Houston was much higher than that at St. Louis, as expected. Also, the outlet concentration at Houston was higher. Considering this, it is somewhat surprising that the inlet and outlet concentrations of fecal Streptococci, total coliform, and fecal coliform obtained from the one 6-hr sample at St. Louis are generally within the range of values obtained at Houston. Even more surprising is the finding that the total counts at St. Louis were higher than the highest value obtained at Houston for both the inlet and the outlet, even though the particulate concentrations were much lower at St. Louis. It is not known whether this was due to the fact that these are different plants, or due to the differences in the bacterial sampling methods.

SECTION 4

AIRBORNE BACTERIAL SAMPLING METHOD RECOMMENDATIONS

Results from the St. Louis and Houston tests and other test data mentioned earlier have provided information from which test method recommendations have been prepared by the ASTM E-38.07 Subcommittee on Health and Safety Aspects of Resource Recovery (3).

Andersen agar plate impactors for determinations of viable particles counts by size were used at both St. Louis and Houston and also in several other studies. MRI would therefore concur with ASTM's E-38.07 preliminary recommendations that this method be used for that purpose. This ASTM subcommittee also recommends that:

- A preseparator be used where dust levels are high
- Trypticase soy agar be used for total counts, with mycostatin to inhibit fungal growth
- Vogel-Johnson agar be used for Staphyococcus aureus Pyogenes var.
- Litman Oxgall be used for fungal counts, with streptomycin to inhibit bacterial growth
- Levine Eosin methylene blue be used for enterics

Also, as pointed out by E-38-.07, the Andersen impactor gives data on the number of particles containing viable cells rather than the number of cells themselves. To an extent this is true of all methods but is more prevalent with the Andersen impactor because it is not designed for dispersing the sample.

For determination of bacteria, irrespective of size, sampling was carried out at St. Louis using a Hi-Vol method. For source sampling, impinger samplers were used at St. Louis and Houston; and in one case at St. Louis, this was supplemented with Hi-Vol sampling. Results from the latter, as discussed earlier, indicated considerably lower values for the Hi-Vol method. Therefore, MRI concurs with E-38.07 that filter methods are not generally recommended because of the dessication effects of the airscream. Alternatively, it is recommended that impinger samplers be used. Since AGI-30 impingers are designed for

microbial sampling, MRI's judgment at this time is that these would be the preferred sampler.

The AGI-30 impinger samplers would also be recommended for testing of source emissions; but where large particles are being emitted by the source, several complications arise. First, traversing of the duct may be necessary along with isokinetic sampling. This raises several problems because the size of the sampling probe nozzle must be large in order not to become plugged with the large particles, and this in turn requires a high sampling rate, above the capability of impingers. A smaller probe could be used with lower sampling rates, but this might require constant attention to the monitoring of the rates, quickly backflushing the probe with air whenever pluggage occurs. This procedure would allow use of the AGI-30 impingers and is probably the best method that can be recommended at this time for sampling source emissions containing large particles.

AGI-30 impingers are suited to ambient or in-plant sampling, and sampling time should be approximately 30 min. Further, E-38.07 recommends that:

- Lactose broth be used for bacterial sampling
- Samples be cooled in ice immediately after being taken.

It must be pointed out, however, that the AGI-30 impingers operate at a relatively low sampling rate and should probably not be operated for periods exceeding 30 min. If longer periods need to be sampled (e.g., 6 hr), a proportionately greater number of samples would be produced for analysis. It is possible, however, that impinger samples could be combined. Also, as mentioned earlier, the nozzle in AGI-30 impingers is quite small and might become plugged, especially during in-plant sampling. If large particles are removed by physical means, such as a preseparator or preimpinger, the actual microbial concentrations will be decreased as the large particles may harbour adsorbed microbes. Therefore, the sampling period should not be extended beyond 30 min, and any material collected in a preseparator or preimpinger should be analyzed for microorganisms.

SECTION 5

VIRUS

Samples taken at St. Louis and Houston were analyzed for viruses, but none were found.

Dr. Peterson (11) has reported average viral concentrations in municipal solid waste (MSW) of 0.32 pfu/g. If one assumes that particles of MSW are suspended in air at a relatively high concentration, on the order of 1,000 $\mu g/m^3$, it can be calculated that the suspended viral concentrations would be only 0.00032 pfu/m^3. Thus, one would have to sample over 3,000 m^3 of air to capture one virus, if the virus remained viable. This quantity is far beyond the practical limits for impingers or Hi-Vols. The reason no viruses were found during the St. Louis and Houston testing could well be an insufficient amount of air was sampled to yield a probability of finding a virus.

Therefore, MRI cannot recommend any suitable sampling technique for viruses; and perhaps there is no need for such a method if the concentrations are as low as the above calculations would indicate, based on the results reported by Dr. Peterson (11).

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